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Published in:
Journal of Bacteriology

Publication date:
1997

JOURNAL OF BACTERIOLOGY, Nov. 1997, p. 6979–6985 Vol. 179, No. 22
0021-9193/97/$04.00+0

Betaine and L-Carnitine Transport by *Listeria monocytogenes* Scott A in Response to Osmotic Signals

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Received 17 June 1997/Accepted 9 September 1997

The naturally occurring compatible solutes betaine and L-carnitine allow the food-borne pathogen *Listeria monocytogenes* to adjust to environments of high osmotic strength. Previously, it was demonstrated that *L. monocytogenes* possesses an ATP-dependent L-carnitine transporter (A. Verheul, F. M. Rombouts, R. R. Beumer, and T. Abee, J. Bacteriol. 177:3205–3212, 1995). The present study reveals that betaine and L-carnitine are taken up by separate highly specific transport systems and support a secondary transport mechanism for betaine uptake in *L. monocytogenes*. The initial uptake rates of betaine and L-carnitine are not influenced by an osmotic upshock, but the duration of transport of both osmolytes is directly related to the osmotic strength of the medium. Regulation of uptake of both betaine and L-carnitine is subject to inhibition by preaccumulated solute. Internal betaine inhibits not only transport of external betaine but also that of L-carnitine and, similarly, internal L-carnitine inhibits transport of both betaine and L-carnitine. The inhibition is alleviated upon osmotic upshock, which suggests that alterations in membrane structure are transmitted to the allosteric binding sites for betaine and L-carnitine of both transporters at the inner surface of the membrane. Upon osmotic downshock, betaine and L-carnitine are rapidly released by *L. monocytogenes* as a consequence of activation of a channel-like activity. The osmolyte-sensing mechanism described is new and is consistent with various unexplained observations of osmoregulation in other bacteria.

Food-borne listeriosis caused by *Listeria monocytogenes* has emerged as a topic of considerable public health concern over the past decade. The infection is encountered in neonates, elderly persons, pregnant women, and the immunocompromised, and symptoms may include sepsis, meningitis, infection of the central nervous system, abortion, and stillbirth, with fatality rates of approximately 25%. The ubiquitous distribution of *L. monocytogenes* in the environment and its relative high tolerance to environmental stresses such as low temperature and high osmotic strength contribute to its status as a hazard in minimally processed ready-to-eat refrigerated products (7).

Cold and salt tolerance in *L. monocytogenes* can be imparted by betaine and L-carnitine. Betaine is present in high concentrations in foods originating from plants, whereas foods of animal origin generally have a high carnitine content (3, 15, 21, 30). The osmoprotective capacity of betaine is well-known among prokaryotic organisms, whereas, so far, L-carnitine has been recognized only as an osmolyte in *L. monocytogenes*, *Lactobacillus plantarum*, and *Escherichia coli* (3, 12–14). The involvement of betaine and L-carnitine in cold tolerance of the psychrotroph *L. monocytogenes* has recently been reported (15, 25, 30). Uptake of L-carnitine in *L. monocytogenes* is mediated by a constitutively expressed transporter that is driven by ATP. Competition experiments revealed that the L-carnitine transporter has a high affinity for carnitine, acetylcarnitine, and γ-butyrobetaine and shows neglible affinity for betaine and L-proline (30). Uptake of betaine in *L. monocytogenes* has been demonstrated to be driven by the electrochemical ion gradient (9, 21).

The present study was initiated to obtain information on the regulation of betaine and L-carnitine accumulation in *L. monocytogenes* in response to changes in the osmolarity of the growth medium. Since bacterial cells not only need to carry out processes to adapt to osmotic upshift, but in addition require ways to excrete intracellular solutes in order to overcome the danger of bursting in case of an osmotic downshock, the release of betaine and L-carnitine by *L. monocytogenes* was studied as well. Stretch-activated channels have been implicated in the fast, nonspecific solute efflux after osmotic downshock in both gram-negative and gram-positive bacteria (2). However, there is increasing evidence for specific osmolyte efflux systems, independent of the uptake system in *E. coli*, *Salmonella typhimurium*, *Ectothiorhodospira halochloris*, and *L. plantarum* (10, 16, 17, 24, 28).

MATERIALS AND METHODS

Bacterial strain and growth conditions. *L. monocytogenes* Scott A cells were grown in a chemically defined minimal medium (DM) as described previously (23). The osmolarity of this medium, measured by freezing-point depression with an Osmomat 030 (Gonotec, Berlin, Germany), was 0.4 osmol/kg. High-osmolarity growth conditions were obtained by addition of NaCl or KCl (0.3 or 0.5 M) to DM. Media were supplemented with betaine or L-carnitine as indicated. Cultures were grown at 37°C with shaking (200 rpm) until the optical density at 660 nm (OD_{660}) reached 0.65 (late-exponential growth phase).

Determination of intracellular osmolyte content. Cells were harvested by centrifugation and washed twice by resuspension and centrifugation in 50 mM potassium phosphate (pH 6.8)–5 mM $MgSO_4$, which was isotonic with the growth medium. The pelleted cells were freeze-dried and extracted by the procedure of Galinski and Herzog (8), which is a modification of the method of Bligh and Dyer (4). Betaine and L-carnitine concentrations were determined by refractive index after high-performance liquid chromatography with a LiChrosphere 100-NH_2, 5-μm column (Merck, Darmstadt, Germany) at a flow rate of 1 ml/min at 45°C, with a mobile phase of 80:20 (vol/vol) acetonitrile–20 mM potassium phosphate (pH 7.0).

Transport studies. Cells were grown until the OD_{660} reached 0.65, washed twice, and resuspended at an OD_{660} of 20 in 50 mM potassium phosphate (pH 6.8) containing 5 mM $MgSO_4$, unless mentioned otherwise, and stored on ice

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until use. Cells (OD_{660} of 1) were preenergized at 30°C with 10 mM glucose for 5 min prior to the addition of radiolabelled substrate (final concentration of 1.3 mM). Potential competitors of betaine and L-carnitine transport were introduced together with the label at an 80-fold excess. The buffer osmolarity was raised by addition of aliquots of 3 M KCl or NaCl or buffer simultaneously with the radiolabelled substrate. Samples were withdrawn, and uptake was stopped by addition of 2 ml of cold LiCl (0.1 to 1 M, depending on the osmolarity of the assay buffer) (11, 13), and the cells were collected on 0.2-μm-pore-size cellulose nitrate filters (Schleicher & Schuell GmbH, Dassell, Germany) under vacuum. The filters were then washed with another 2 ml of LiCl, and the radioactivity trapped in the cells was measured with a liquid scintillation counter (model 1600TR; Packard Instruments Co., Downers Grove, Ill.). Uptake of osmolytes was normalized to total cellular protein, which was determined by the method of Lowry et al. (19), with bovine serum albumin as a standard. A specific cytoplasmic volume of 3 μl per mg of cell protein was used for the calculation of the internal substrate concentration (20).

Osmotic downshock experiments were performed with cells that had accumulated radiolabelled substrate in the presence of 0.8 M KCl; these cells were diluted fivefold with 50 mM potassium phosphate (pH 6.8)–5 mM $MgSO_4$ containing radiolabelled substrate (preheated at 30°C). Exchange reactions were assayed by allowing the cells to accumulate radiolabelled substrate until the steady state was reached (60 to 70 min). Subsequently, competitors or inhibitors were introduced, and samples were removed at time intervals and treated as described above. To monitor the effect of internal osmolyte pools on betaine and L-carnitine uptake, cells were loaded for given time periods with 1 or 2 mM unlabelled betaine or L-carnitine in the presence of 10 mM glucose in 50 mM potassium phosphate (pH 6.8) plus 5 mM $MgSO_4$. Cells were washed three times, and the transport of L-[^{14}C]carnitine and [^{14}C]betaine in these cells was determined as described above; the washing procedure did not result in large losses (less than 5%) of intracellular betaine or L-carnitine.

Chemicals. L-[*N-methyl*-^{14}C]carnitine (50 to 62 mCi/mmol) was obtained from Amersham (Little Chalfont, Buckinghamshire, United Kingdom). Radiolabelled betaine was prepared enzymatically as described by Landfald and Strøm (18) from [*N-methyl*-^{14}C]choline (55 mCi/mmol; DuPont, NEN) or [*N-methyl*-^{3}H]choline (60 to 90 Ci/mmol; DuPont, NEN). The identity of radiolabelled betaine was confirmed by thin-layer chromatography (26). Other chemicals were reagent grade and were obtained from Sigma Chemical Company, St. Louis, Mo., or other commercial sources.

RESULTS

Betaine and L-carnitine are transported by separate systems. Transport of [^{14}C]betaine and L-[^{14}C]carnitine was studied in the presence of an 80-fold excess of unlabelled analogs (betaine, L-carnitine, L-proline, dimethylglycine, or tetramethylglycine). Nonradioactive betaine, at an 80-fold excess, completely inhibited [^{14}C]betaine uptake (1.3 mM, final concentration), whereas none of the other compounds had a significant effect on betaine uptake. Similar results were obtained for the transport of L-[^{14}C]carnitine, which was inhibited only by an 80-fold excess of unlabelled L-carnitine (data not shown).

The addition of an 80-fold excess of unlabelled betaine caused the efflux of [^{14}C]betaine from cells that were preloaded with [^{14}C]betaine (Fig. 1A). The potassium proton exchanger nigericin (2 μM) and the potassium ionophore valinomycin (1.5 μM), which in combination dissipate the proton (and, indirectly, the sodium) motive force, also caused exit of [^{14}C]betaine from preloaded cells. Addition of unlabelled L-carnitine to cells that had accumulated [^{14}C]betaine was without effect (Fig. 1A). Preaccumulated L-[^{14}C]carnitine was not released upon the addition of either unlabelled L-carnitine or unlabelled betaine, nor was it released upon the addition of nigericin plus valinomycin (Fig. 1B). These data are consistent with a secondary betaine transport system (9, 21) and an L-carnitine transport system that is dependent on ATP (30). Taken together, these data also show that betaine and L-carnitine enter the cytoplasm via distinct transporters in *L. monocytogenes* Scott A.

Effect of osmolarity on betaine and L-carnitine uptake. Addition of increasing concentrations of KCl to the assay buffer (50 mM potassium phosphate, 5 mM $MgSO_4$) did not significantly influence the initial rates of uptake of betaine and L-carnitine in *L. monocytogenes* Scott A cells grown in DM with 0.3 M KCl (Fig. 2). Lowering the osmolarity of the assay buffer by reducing the potassium phosphate concentration to 10, 5, or 1 mM had no effect either (data not shown). The final levels of both betaine and L-carnitine accumulated were dependent on the osmotic strength; the effect was more pronounced for betaine (Fig. 2). Comparable results were obtained when NaCl or sucrose was used to raise the osmolarity of the incubation mixture (data not shown). The initial rates of betaine and L-carnitine uptake of cells grown in DM were similar to those of cells grown in DM with 0.3 M KCl (data not shown); the latter result excludes significant induction of expression of the genes involved in the uptake of these compounds.

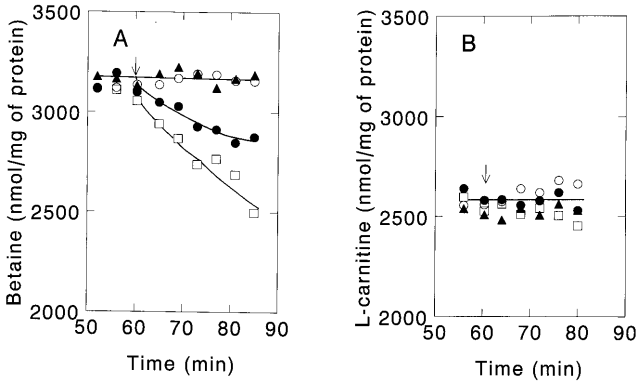

FIG. 1. Betaine and L-carnitine efflux from *L. monocytogenes* Scott A. Cells were grown in DM with 0.3 M KCl, washed, and resuspended in 50 mM potassium phosphate (pH 6.9) containing 5 mM $MgSO_4$. Cells were energized with 10 mM glucose at 30°C and allowed to take up [^{14}C]betaine (A) or L-[^{14}C]carnitine (B) at high osmolarity (0.8 M KCl). Osmolytes were present at a final concentration of 1.3 mM. After 60 min of loading (indicated by arrow), a 80-fold excess of unlabeled betaine (●) or L-carnitine (▲), 1.5 μM valinomycin plus 2 μM nigericin (□), or an equivalent volume of water (○) was added.

Kinetic properties of betaine uptake. Kinetic parameters of [^{14}C]betaine uptake in *L. monocytogenes* Scott A cells grown in DM containing 0.3 M KCl were determined at substrate concentrations of 1 to 2,000 μM in 50 mM potassium phosphate–5 mM $MgSO_4$. Michaelis-Menten and Eadie-Hofstee plots indi-

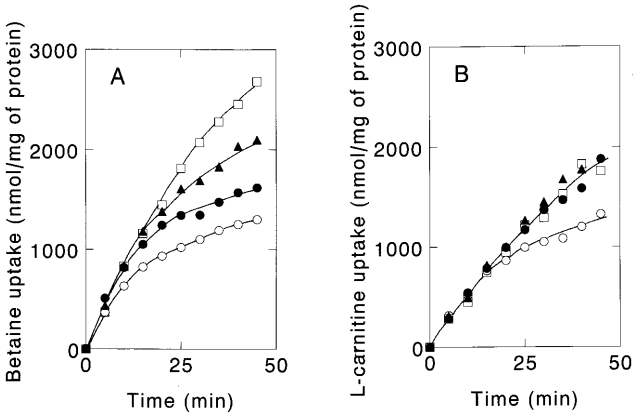

FIG. 2. Effect of osmotic strength on betaine and L-carnitine uptake in *L. monocytogenes* Scott A. Cultures were grown in DM containing 0.3 M KCl. Transport assays of [^{14}C]betaine (A) and L-[^{14}C]carnitine (B) were performed at 30°C in 50 mM potassium phosphate (pH 6.9) containing 5 mM $MgSO_4$. After preenergization with 10 mM glucose for 5 min, KCl was added at various concentrations together with the radiolabelled substrate (1.3 mM, final concentration). ○, No additions; ●, 0.15 M KCl; ▲, 0.3 M KCl; □, 0.6 M KCl.

TABLE 1. Intracellular concentrations of osmolytes in *L. monocytogenes* Scott A cells[a]

Medium addition(s)	Concn (nmol mg of protein^{-1}) of osmolyte in Scott A cells			
	DM grown		DMS grown	
	Betaine	L-Carnitine	Betaine	L-Carnitine
None	0	0	0	0
1 mM Betaine	560 ± 60	0	1,300 ± 140	0
1 mM L-Carnitine	0	640 ± 70	0	990 ± 120
1 mM Betaine + 1 mM L-carnitine	490 ± 50	240 ± 30	1,230 ± 140	110 ± 20
1 mM Betaine + 0.1 mM L-carnitine	490 ± 50	220 ± 30	1,350 ± 140	60 ± 10
0.1 mM Betaine + 1 mM L-carnitine	200 ± 30	400 ± 50	540 ± 60	460 ± 50

[a] Cells were grown until the late-exponential growth phase in DM or in defined medium supplemented with 0.5 M NaCl (DMS), containing betaine and/or L-carnitine as indicated. Values are the means ± standard errors of triplicate samples of independent cultures.

cated an apparent K_m value of 10 ± 2 μM and a V_{max} of 56 ± 8 nmol min^{-1} mg of protein^{-1}. Similar values were obtained under hyperosmotic conditions (50 mM potassium phosphate–5 mM $MgSO_4$ plus 0.6 M KCl). For comparison, L-carnitine was taken up with an apparent K_m of 10 ± 2 μM and a V_{max} of 48 ± 9 nmol min^{-1} mg of protein^{-1} in *L. monocytogenes* Scott A cells (30), irrespective of the osmolarity of the medium.

Coaccumulation and transport of betaine and L-carnitine. To establish a possible preference in the accumulation of either betaine or L-carnitine, the intracellular concentrations were determined for cells grown in DM and DM plus 0.5 M NaCl containing betaine and/or L-carnitine. The addition of 1 mM betaine to DM resulted in an intracellular betaine concentration of 560 ± 60 nmol mg of protein^{-1}, which increased to 1,300 ± 140 nmol mg of protein^{-1} in the presence of 0.5 M NaCl (Table 1). The values obtained in the case of L-carnitine were 640 ± 70 and 990 ± 120 nmol mg of protein^{-1}, respectively. When betaine and L-carnitine were supplied together (both at a concentration of 1 mM), betaine was preferentially accumulated, particularly in the high-osmolarity medium. No difference in the intracellular betaine and L-carnitine pools was detected when the available L-carnitine was reduced from 1 to 0.1 mM. However, a 10-fold reduction in the external betaine concentration (from 1 to 0.1 mM), with L-carnitine present at 1 mM, resulted in a substantial increase in the intracellular L-carnitine pool at the expense of intracellular betaine. Under these conditions, the external betaine pool was not completely exhausted, since the maximal attainable intracellular betaine concentration at an external concentration of 0.1 mM is about 800 nmol mg of protein^{-1}. The finding that the amounts of betaine and L-carnitine accumulated in cells grown in the presence of these osmolytes are about twofold lower than those found under steady-state conditions in our uptake assays is probably due to the presence of amino acids in the defined medium. It has been demonstrated that amino acids can be accumulated to substantial levels in *L. monocytogenes* during growth at high osmolarity (1, 20).

The relationship between regulation of betaine and L-carnitine accumulation and dependence on osmotic stress was further investigated by analyzing betaine and L-carnitine transport simultaneously in a dual-label experiment. At low osmolarity, initial uptake rates of betaine and carnitine were similar, whereas over the course of time, betaine was accumulated to a higher level than L-carnitine (Fig. 3). Upon osmotic upshock, the initial rates of uptake remained the same (approximately 45 nmol min^{-1} mg of protein^{-1} [data not shown]), but the accumulation of betaine and L-carnitine reached in the pools eventually increased. The increment in the final level of betaine was particularly prominent in the high-osmolarity medium.

Internal betaine and L-carnitine pools at different external osmolarities. In an earlier study (30), we observed that growth in the presence of L-carnitine or betaine reduced the rate of L-carnitine transport, which could be explained by an inhibition of the L-carnitine transport system by intracellular betaine and L-carnitine. These considerations, together with the apparent lack of a direct effect of osmolarity on carrier activity, prompted us to study the regulation of the L-carnitine and betaine transport systems by internal (preaccumulated) substrate in detail. Cells were grown in DM with 0.3 M KCl lacking betaine and L-carnitine, collected at the late-exponential growth phase, washed two times, and subsequently incubated with unlabelled betaine or L-carnitine at a final concentration of 1 mM for different periods of time in the presence of glucose to obtain cells that were preloaded with the osmolytes to different levels. The exact values of these internal levels were assessed in control experiments in which cells were incubated with radiolabelled betaine or L-carnitine under the same experimental conditions. Following the removal of external betaine or L-carnitine, the initial rate of [^{14}C]betaine uptake as a function of internal osmolyte concentration was determined. As shown in Fig. 4, the rates of uptake of betaine (Fig. 4A) and L-carnitine (Fig. 4B) decreased with increasing internal amounts of betaine and L-carnitine. This suggests that both transporters are subject to inhibition by preaccumulated be-

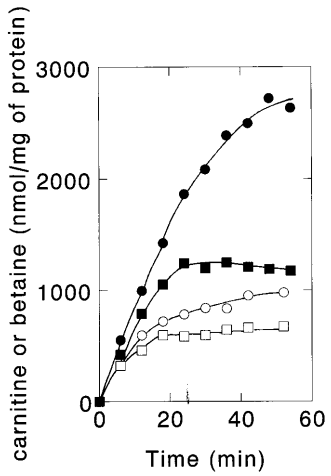

FIG. 3. Simultaneous uptake of [^{3}H]betaine and L-[^{14}C]carnitine under low- and high-osmolarity conditions. Cells were grown in DM containing 0.3 M KCl, washed, and resuspended in 50 mM potassium phosphate (pH 6.9) containing 5 mM $MgSO_4$. After 5 min of preincubation with 10 mM glucose at 30°C, [^{3}H]betaine plus L-[^{14}C]carnitine (final concentrations of 1.3 mM) were introduced with (●, ■) or without (○, □) 0.8 M KCl. Transport of [^{3}H]betaine (○, ●) and L-[^{14}C]carnitine (□, ■) is depicted.

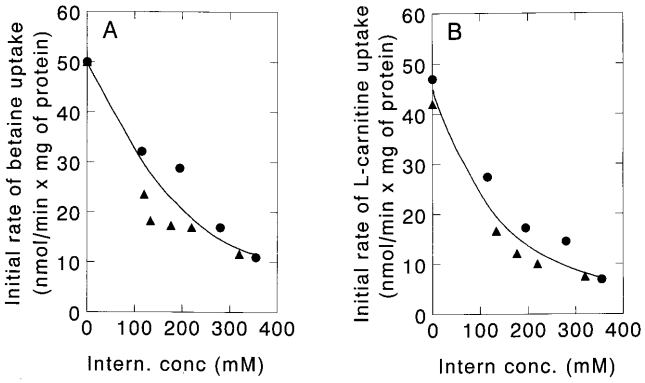

FIG. 4. Rates of betaine and L-carnitine uptake at different internal osmolyte concentrations. Cells grown in DM with 0.3 M KCl were washed and resuspended in 50 mM potassium phosphate (pH 6.9) containing 5 mM $MgSO_4$. To establish different internal osmolyte concentrations, the cells were preloaded at 30°C in the presence of 1 mM betaine or L-carnitine and with 10 mM glucose as an energy source. The rates of uptake of [^{14}C]betaine and L-[^{14}C]carnitine were estimated from levels of uptake for 10 min in the presence of 10 mM glucose and after removal of unlabelled substrate. ●, internal betaine concentration; ▲, internal L-carnitine concentration.

taine and L-carnitine and that betaine and L-carnitine are equally effective in inhibiting the activity of the transporters at the inner site of the cytoplasmic membrane.

Next, cells that had been preloaded with unlabelled betaine or L-carnitine for 30 min, resulting in internal concentrations of approximately 350 mM, were used to study uptake of [^{14}C]betaine and L-[^{14}C]carnitine at various external osmolarities. Without osmotic upshock, the rates of uptake of [^{14}C]betaine and L-[^{14}C]carnitine in cells preloaded with betaine or L-carnitine, respectively, were low (about 10 nmol min^{-1} mg of $protein^{-1}$) (Fig. 5). A significant increase in the initial rates of uptake of both [^{14}C]betaine and L-[^{14}C]carnitine transport was encountered upon imposition of hyperosmotic conditions on cells preincubated with betaine and L-carnitine, respectively (Fig. 5). Similar results were obtained when NaCl instead of KCl was used to raise the osmolarity of the assay mixture (data not shown). The insets of the figures show that hyperosmotic conditions had no effect on the initial rate of uptake when betaine and L-carnitine are not present intracellularly. These data suggest involvement of membrane tension or turgor pressure in the activity of the transporters, which seems to exert its effect on the interaction of the ligand (substrate) with the carrier protein at the *trans* site of the membrane.

Betaine and L-carnitine exit upon osmotic downshock. When glucose-metabolizing cells that had accumulated betaine or L-carnitine were subjected to an osmotic downshock, rapid efflux of both osmolytes was observed (Fig. 6). These fast effluxes of betaine and L-carnitine were completed within 5 s. In addition, a second, slower phase of efflux was noticeable, which was particularly prominent for betaine.

DISCUSSION

In this study, it was shown that *L. monocytogenes* Scott A possesses highly specific and separate transport systems for the osmolytes betaine and L-carnitine. The two transporters have comparably high affinities for their substrates (apparent K_ms of about 10 μM). These findings are unique, because both transporters have immense scavenging capacity for their substrate, and specific independent transport systems for betaine and carnitine have not been described for other microorganisms. In

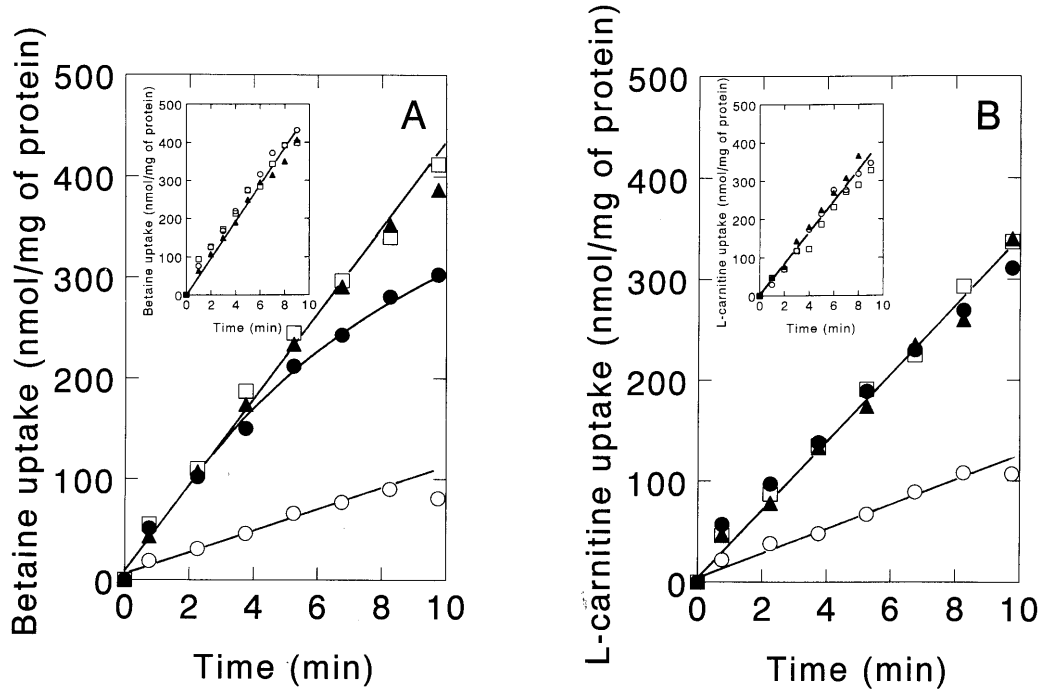

FIG. 5. Effect of internal osmolytes and hyperosmotic conditions on transport of [^{14}C]betaine and L-[^{14}C]carnitine in *L. monocytogenes* Scott A. Cells were grown in DM with 0.3 M KCl, washed, and resuspended in 50 mM potassium phosphate (pH 6.9) containing 5 mM $MgSO_4$. Aliquots of cells were preloaded with unlabelled betaine or L-carnitine for 30 min as described in the legend to Fig. 3. Following the removal of unlabelled substrate, uptake of radiolabelled substrates was assayed at various external osmolarities. Uptake of [^{14}C]betaine was conducted with cells preincubated with betaine (A), and uptake of L-[^{14}C]carnitine was performed with cells preincubated with L-carnitine (B). In the insets, the rates of uptake of betaine and L-carnitine in nonloaded cells are shown for comparison. ○, no additions; ●, 0.15 M KCl; ▲, 0.3 M KCl; □, 0.6 M KCl.

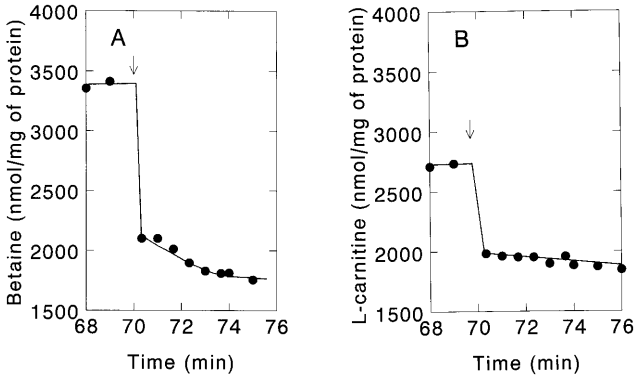

FIG. 6. Efflux of betaine and L-carnitine from *L. monocytogenes* Scott A cells in response to osmotic downshock. Cells grown in DM with 0.3 M KCl were washed and resuspended in 50 mM potassium phosphate (pH 6.9)–5 mM $MgSO_4$. After 5 min of preenergization with 10 mM glucose at 30°C, uptake was initiated by addition of 1.3 mM [^{14}C]betaine (A) or 1.3 mM L-[^{14}C]carnitine (B) together with KCl (final concentration of 0.8 M). After 70 min of uptake, samples were diluted fivefold with 50 mM potassium phosphate (pH 6.9) containing 5 mM $MgSO_4$ plus 1.3 mM [^{14}C]betaine (A) or 1.3 mM L-[^{14}C]carnitine (B).

L. plantarum, betaine and L-carnitine enter the cytoplasm via the same transport system, with high affinity for both substrates (10), and in *E. coli*, carnitine is transported via ProP and ProU, which are driven by the electrochemical ion gradient and ATP, respectively (6, 29).

In a previous study, we demonstrated that in *L. monocytogenes* Scott A, L-carnitine is taken up via an ATP-dependent transporter (30). In a recent study by Gerhardt et al. (9), evidence was obtained for the involvement of a sodium motive force as the driving force for betaine uptake in *L. monocytogenes* DP-L1044. The addition of unlabelled betaine to *L. monocytogenes* Scott A cells, preloaded with [^{14}C]betaine, resulted in the exit of radioactive label, which is thought to be due to exchange of internal for external betaine. Moreover, dissipation of the electrochemical ion gradients by nigericin plus valinomycin to cells that had preaccumulated [^{14}C]betaine caused efflux of label driven by the betaine concentration gradient. These results support a secondary transport mechanism for betaine uptake in *L. monocytogenes* Scott A, because these systems generally operate in two directions in contrast to ATP-dependent solute uptake systems.

Strikingly, we did not find any evidence for osmolarity- and/or turgor-controlled activity of the betaine and L-carnitine transporters in *L. monocytogenes*. Neither of the transporters is significantly induced in a high-osmolarity environment, and the initial rates of uptake are not affected by an osmotic upshock. Osmotic stimulation is a feature of many compatible solute transporters in bacteria and is regarded as indicative of a role of these transporters in adaptation to low-water-activity environments (5, 6, 10, 16, 17). However, we demonstrated that both transporters in *L. monocytogenes* are subject to other control mechanisms. The final levels of accumulation of betaine and L-carnitine were influenced by the medium osmolarity, and, in addition, it was demonstrated that regulation of uptake of both betaine and L-carnitine is subject to inhibition by preaccumulated solute. Internal betaine not only inhibited the transport of external betaine, it additionally inhibited the transport of external L-carnitine. Accordingly, internal L-carnitine inhibited the transport of both betaine and L-carnitine. This represents a novel mechanism of regulation of compatible solute accumulation, i.e., both transporters are inhibited by the presence of compounds on the inner surface of the membrane which are not recognized by the respective transporter at the outer surface of the membrane. We therefore propose that there is an allosteric binding site on the internally exposed regions of both transporters accepting betaine as well as L-carnitine (Fig. 7). The inhibition of betaine and L-carnitine transport by preaccumulated solute was relieved when cells that had been preloaded with betaine or L-carnitine were subjected to an osmotic upshock. This is most likely a consequence of an altered membrane structure due to changes in membrane tension affecting conformation of the transporters. In kinetic terms, it could mean that upon osmotic upshock, the affinity of

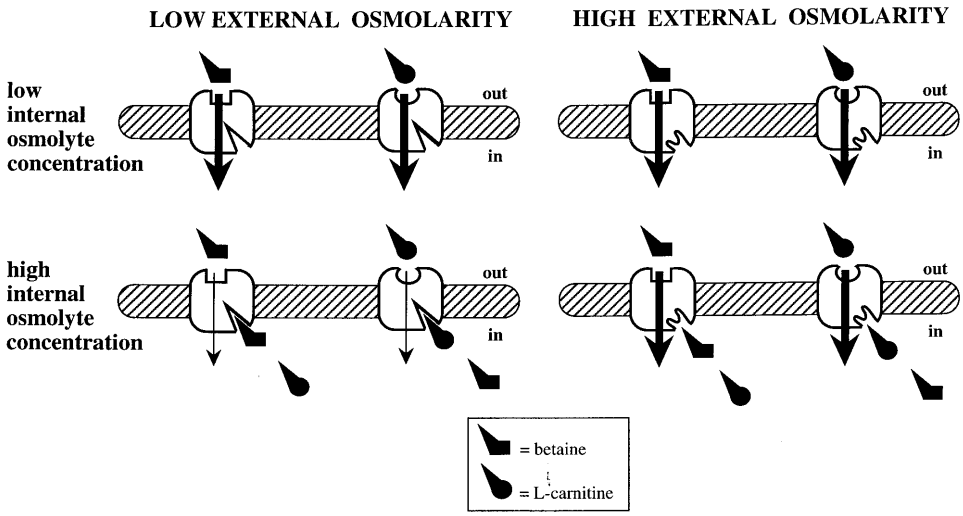

FIG. 7. Model for osmotic regulation of betaine and L-carnitine uptake in *L. monocytogenes* Scott A. Different conformations of the putative betaine and L-carnitine uptake systems are depicted for low- and high-osmolarity media and under conditions in which the intracellular concentrations of betaine and/or L-carnitine are low and high; the thickness of the arrows reflects the relative activity of the transport systems under the various conditions. The intracellularly exposed allosteric binding sites of transporters that recognize both betaine and carnitine allow control of osmoprotectant accumulation. Upon hyperosmotic shock, transport of betaine and L-carnitine in *L. monocytogenes* Scott A is stimulated in proportion to the substrate concentration at the *trans* site of the cytoplasmic membrane. Without preaccumulated substrate, the activity is already maximal and is not affected by an osmotic upshock.

the allosteric binding site of the transporters for betaine and L-carnitine at the inner surface of the membrane is lowered (Fig. 7).

L. monocytogenes cells cultured in DM supplemented with 1 mM betaine plus 1 mM L-carnitine accumulated about a two-fold-higher intracellular level of betaine than L-carnitine. The preferred accumulation of betaine was even more prominent in a high-osmolarity environment. The observation that the L-carnitine transport system is inhibited more strongly by internal betaine or L-carnitine (50% inhibition at 110 mM) than the betaine transport system (50% inhibition at 175 mM) may explain the preferred uptake of betaine over L-carnitine. From the dual-label uptake experiment, it could also be inferred that the initial rates of uptake of betaine and L-carnitine (no internal osmolytes present yet) were not affected by the extracellular presence of the other substrate.

The initial rates of uptake in preloaded cells were similar irrespective of whether the osmotic strength was raised by 0.15, 0.3, or 0.6 M KCl (or NaCl) and were comparable to those obtained with nonloaded cells under the same osmotic stress conditions. These data may explain why Patchett et al. (21) observed much lower betaine uptake rates in *L. monocytogenes* NCTC 7973 (5 to 10 times lower) than we did in *L. monocytogenes* Scott A. Their studies were conducted with washed cells following growth in broth, which is known to contain significant amounts of betaine and carnitine (14, 15, 22, 25, 27). Accumulation of these compatible solutes during growth could have resulted in the reduced uptake rates for betaine. The approximate 10-fold-increased uptake rate in the presence of NaCl is consistent with the regulation of activity by an osmotic upshock as described in this paper.

When *L. monocytogenes* was faced with a decrease in the external osmolarity, i.e., a hypotonic osmotic shock, immediate release (within 5 s) of the osmolytes betaine and L-carnitine was observed. The rates of efflux were much higher than the rates of uptake, which suggests that this rapid efflux of betaine and L-carnitine is mediated via a channel-like activity rather than via a carrier protein (2, 10). The rapid release of osmolytes upon osmotic downshock was followed by a slower second phase of efflux, which was particularly significant in the case of betaine. This is most likely due to downhill efflux of [^{14}C]betaine via the same carrier protein that is responsible for the uptake of betaine. A similar pattern of biphasic efflux of betaine was reported for *L. plantarum* cells that were loaded with [^{14}C]betaine. In this organism, betaine is taken up via a kinetically irreversible ATP-dependent transport system, and a distinct uniport system was proposed for the slow phase of betaine efflux upon an osmotic downshock (10).

The experiments presented in this paper also show that *L. monocytogenes* not only establishes substantial internal concentrations of betaine and L-carnitine when grown at high osmolarity (i.e., DM containing $\geq$0.15 M NaCl or KCl), but also accumulates significant amounts of betaine and L-carnitine when grown in DM (0.4 osmol/kg) without added NaCl or KCl. Accumulation of compatible solutes in the absence of osmotic stress has also been described for other gram-positive bacteria. This is thought to be a direct consequence of their maintenance of a relatively high turgor pressure compared to that of gram-negative bacteria (5, 11, 27, 31).

The most important findings of the present study relate to the osmotic regulation of betaine and L-carnitine uptake, which are summarized in Fig. 7. The osmolyte-sensing mechanism in *L. monocytogenes* is different from that in other microorganisms, including *E. coli*, *S. typhimurium*, and *L. plantarum*, in which an osmotic upshock activates the system irrespective of the internal substrate concentration (10, 16, 17).

ACKNOWLEDGMENT

This study was supported by a grant from the EC (EC-AIR 1-CT92-0125).

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